

β -D-galactoside.

Nov. 10, 1947.

Sample from E. coli (2 grams).

Test in comparison with lactose + galactose at .05% in T (m).

Add necessary growth factors.

galactose^(A), lactose^(B), β -D-galactoside^(C), β -D-galactose^(D).

	1	58-161	2	Y87.	3	W-30.	4	W-35	5	W-36.	6	Y10	7	Y53.	8	W-2.
	+	++	-	⊕	++	±	±	-	+	++	+	++	+	++	-	-
	+	++	-	⊕	++	-	-	-	-	-	+	++	+	++	-	-
	-	-	-	-	++	-	-	-	-	-	-	-	-	-	-	-
	++	++	✓	±	±	++	-	-	-	-	++	++	+	++	-	-
	++	++	✓	±	±	±	-	-	-	-	-	-	-	-	-	-
	++	++	++	++	++	++	-	-	-	-	++	++	±	++	++	✓
	++	++	-	±	±	+	-	-	-	-	++	++	+	++	-	-
	±	++	++	++	++	-	-	-	-	-	++	++	±	±	-	-

Readings at 20h., 24h., 36h.

ϕ -galactoside is not generally utilized and may be slightly inhibitory in galactose media. Cf Y10 however.

56 hours; 72 h.

	gal	lac	β -D-gal	β -D-gal + gal.	
1	++	++	++ /	++	
2	++	++	- ✓	++	
3					
4	++	++	- ✓	++	
5	++	++	- ✓	++	
6	++	++	+ ± ✓	++	
7	++	++	++ ✓	++	
8	++	++	-	++	

Lac + cells present

Note that none of those cultures originally lac- have grown on β -D-galac.

Considerable pigment produced
in galactose

Nov 15 1947

Inocula from 23 SP15. 0.1 ml/tube T(BMTLB1) base.

A (Galactose .05%)

B (β -Galactoside)C Galactose + Phenol
.02%

TIME::: 5P16

Inoculum		
1	{ gal	1a
2	{ lac	1b
3	{ lac	1c
4	{ gal	2a
5	{ lac	2b
6	{ gal	7a
7	{ lac	7b
8	{ β -d	7c
9	{ gal	8a
10	{ lac.	8b

5P16

++
++
(+++)
-
++
±
++
(+++)
-
++

5P16.

++
++
++
++
++
++
++
++
+++
++

?? Is utilization of β -galactoside by wild type mutants?

SP17

on gentiobiose +

+

"α- β -galactoside" +

++

7a on gentiobiose +

+++

"d- β -galactoside" +

++

P17. Strains on β -galactoside EMB:

1A; 1C, 1B.

6A; 6C.

A19. 1: all show a slow type of colony & a few multi-layered suggestive of rapid utilization. 1B and 1C show these particularly. all streaks are papillated.

6: somewhat smeared. Two colony types also noted.

Need checking in phenol + galactose.

Nov. 27, 1947

Test on EMB agar using heavy water suspensions of cells from YP agar slants, except W-28 and W-29 from galactose EMB agar.

48 hr. readings.

	W33	+++	W35	-		
	W37	++	W36	-		
	W38	++	Y70	++		
1. K12.	++	W41	W40	++	Y53	++
2. Y10	++	W28	W42	++	Y87	++
3. 58-161	++	W29	W43	-	W30	++
4. W53	+++	W44	W45	-	W53	+
		W46	W48	-		
		W50	W49	-		
		W51	W-1	+++		

24 hrs. (A29) W52 + All others -

36 hrs. W52 +++ W-1, W33 ++, Y10 +, Y70, Y53 + W53: -

48 hrs. 60 hrs. As above?

There seems to be a graded spectrum of responses. Y52, W-1, W-51 and W-33 are distinctly the most positive reactors, especially W52. The "negative" types are all "sectorial" mutants derived from 58-161 and are Lac negative. Since their Lac+ counterpart is $\beta\phi+$ a relationship is suggested! The only strain which is even relatively "Lac+ $\beta\phi-$ " is W53. while Y53 is Lac- $\beta\phi+$.

Note: Lac+ Lac-

~~Lac- $\beta\phi+$~~ Y10 Y53, W-1. $\beta\phi-$ W53 W45, -49.

Suggested Crosses. W53 x W-1 Lac+ $\beta\phi-$ x Lac- $\beta\phi+$, also Mal+/-
W45 x Y10 Lac- $\beta\phi-$ x Lac+ $\beta\phi+$.

Trehalose/Maltose Ceas adaptatio*n*, pedum.

Dec. 10, 1947.

Prepare 10% suspensions of

- a. Y40 Lac+
- b. W-1 Lac,-
- c. W-45 Lac₂-

Inc. in 37° water bath

Add 1 ml bacteria to 1 ml 4% lactose + dil. to 5 ml. Use Durham tube for gas, and BCP for acid production. Do mixtures in duplicate. + reflux to acid production. (.1 ml M/10 buffer pH 1.0 added.) Set up. 3:45 P.M.

1. a	—	+++
2. b.	—	—
3. c.	—	—
4. a+b	—	+++
5. a+c	—	++
6. b+c.	—	—
a glucose	+++	++
c glucose	++	+++

Mixtures of Lac,- and Lac₂- therefore cannot ferment lactose.

Adaptation takes some time under these conditions. (No extra N)

Dec. 11.

For ~~the~~ Trehalose, use culture of Exp 25 and compare w/ glucose adapted from same culture. (Controls are inadquate.) Set up 4:15 P.M.

Brown in	Trehalose
A glucose	glucose
B " "	maltose
C Trehalose	glucose
D " "	maltose

TREHALOSE***MALTOSE CROSS-ADAPTATION EXPERIMENT.

Dec. 16, 1947.

Grow K-12 in T₆₀) plus .05% sugar 24 h. Harvest and concentrate to ca 10^{10} /ml/

Add 1 ml. cells to 1 ml 5% sugar, and in replicates add NaN_3 to a final conc. of 2×10^{-3} M. Add 0.1 ml M/10 phosphate buffer pH 7.0 and ,05 ml BromCresolPurple .15%

Make up to 5 ml with water, cells added 2 P 16, incubate in 37° water bath.

Readings at 2 h., 4 h., and 18 h., Readings - unless indicated.

Celloglucosm: 2h. 4h. 18h.
4P17 6P17 10A18

Set up. 2P17

- A. Glucose } T(0) + ,05% sugar 18 hours.
- B. Maltose } Harvest + concentrate.
- C. Trehalose }

A. Gluc.
" + Azide

+++ +++
± ±
- -

A cells did not adapt in 18 hrs. in presence of azide, either to trehalose or to maltose.

M
" + A₂

- -
- -

B cells utilized maltose in the presence of azide, but did not adapt to trehalose.

T_r
" + A₂

- -
- -

C cells utilized maltose as well as trehalose and glucose, even in presence of maltose.

B. G
" + A₂

+++ +++
- -
+ +

Azide in conc. of 2×10^{-3} M does inhibit fermentation to some extent but seems to block adaptation completely.

M
" + A₂

- -
- -
+ +

Conc. trehalose and maltose cross-adapt, but only unilaterally, trehalose adaptation implying maltose adaptation, but not the converse.

C G
" + A₂

+++ +++
- -
+ +

Query: Will malt-(Tre-) cells utilize maltose if grown on trehalose?

M

M + A₂

T_r

T_r + A₂

+ +
- -
± ±
- -

Azide does seem to interfere with the fermentation as well as adaptation. T_r-adapted seem to be maltose adapted but not vice versa

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The inhibition of lactose-adaptation
by Azide.

Dec. 18, 1947.

Harvest K-12 from YP-.1%glucose broth. 16 hr. cultures. Conc. 50/20.

Tubes contain in 3 ml. : 1% sugar, 1 ml cells, .1ml Phosphate Buffer M/10 pH 7.0 and indicated conc. azide or DNP Set up 12:20 PM

Glucose Azide M/100 X	(3:20)		21.4. 3:40PM. 6:00PM	Lactose 9A20. 3:40	21.4. 6:00PM. 7:00PM		21.4. - (pH)	
	3:40PM.	6:00PM			9A20.	3:40	6:00PM	7:00PM
1. -	+++	-		✓ 4.50 -	+	++	+++	4.62
2. 1	++	✓		✓ 5.79 -	-	-	-	6.28
3. .5	+±	++		✓ 5.57 -	-	-	+	5.95
4. .1	++	✓		✓ 4.78 -	±	+	++	5.48
5. .05	+++	✓		✓ 4.70 -	+	++	+++	5.18
6. .01	+++	✓		✓ 4.36 -	+	++	+++	5.01
DNP 10^{-4} M X								
7. 5	-	✓		✓	-	-	-	
8. 1	++	✓		✓	-	-	-	
<i>original solution</i> At 12:40, none changed.								7.36

DNP itself is an indicator. 10^{-3} M azide does not appreciably inhibit fermentation,
but it does permit slight adaptation:

The pK of phosphate buffer is 7.21. $pH = pK + \frac{(\text{base})}{(\text{acid})}$

At the initial pH the ratio is ca. 1.6 : 1 There are altogether 10 mM phosphate. At pH 4.50, the ratio is 1:50. The lower the pH, the more sensitive the pH is to slight additions of acid. i.e. all but 2% of the base is reacted, and about 6 mM H⁺ have been produced (from 30 mg = $\frac{1}{6}$ mM = 167 mM glucose). More buffer should be used in this system and an indicator used whose pH is near the pK of phosphate such as bromothymol blue.

On the maltose activity of trehalase.

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Dec. 18, 1947.

W-34.

Grow ~~W-34~~ in T(RO₂) + .1% trehalose and glucose. No growth (\pm) or Test for activity on glucose and maltose in system like Exp. 6 S. Harvest 50 ml & conc. to 2 ml. 50/2. Set Up. SP 19.

Growing conditions -

2h. Glucose
7P19 9A20

Maltose

Glucose +++ +++

- -

Trehalose. \pm +++

- -

W-1 is therefore capable of producing trehalase but not maltase.

So far, all Mal- mutants are apparently Tre+, although W-21 is perhaps a little slow on trehalose.

Maltase is not simply an incidental activity of trehalase.

Cross-adaptation of galactosides

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Jan. 14, 1948.

Harvest cells from 1% cultures in T(m) 36 h. into 1 ml. (K-12)

Set up tests with 1 ml cells, 1 ml 3% substrate, M/200 Hzide and 1 ml M/10 phosphate BCP indicator.

Substrates: G, glucose; L, lactose; M, b-methylgalactopyranoside; and B, N-Butyl-b-galactopyranoside., Ga, galactose.

Grown in/tested on:

Set up 11A, 37°.

G/GA	G/G	G/L	G/M	G/B	L/G	L/L	L/M	L/B	L/Ga
-	±	—	—	—	—	—	—	—	—
5PM 10A 15. (23h.)	—	++	—	—	+±	++	+	—	±

M/G	M/L	M/M	M/B	B/G	B/L	B/M	B/B
±	+	±	—	±	±	—	—
+++	+++	+++	±	+++	+++	+	±

Tested →

Glucosid	Glucose	Lactose	Butyl-gal.	Methyl-gal.	Galactose
Glucose	+++	—	—	—	—
Lactose	±	±	—	+	±
Butyl--	+++	+++	±	+	—
Methyl--	+++	+++	±	+++	—

Cells probably too old for rapid adaptation. Lactose cells in especially conditions.

In future, use mixture of BCP and BTB or most marked contrasts.

Use 2 BTPB: 1 BCP.

Cells may be too old.

(Contd.) ① M adapted are L adapted. ② L adapted are M adapted

③ B is poorly utilized under these conditions! ④ Galactose is a adaptive

⑤

Utilization of C-sources

Jan. 23, 1948.

Grow W-108, Y87, W56 and Y10 in YB broth overnight. Use $\frac{1}{2}$ ml inocula into 10 ml. indicator broth with 1% sugar.

	Maltose		lactose		
108	-	-	-	-	+
108	-	-	-	-	++
87	+++	/	/	-	-
87	+++	/	/	-	-
56	±	/	/	+++	-
56	±	/	/	+++	-
108;56	±	/	/	+++	adapted
108;56	±	/	/	+++	much easier
108;87	1		-	-	-
108;87			-	-	-
Y10	+++	/	/	+++	-
Y10	+++	/	/	+++	-

By P25 all +++ except W56/W..

"herefore, W108 cells do not produce maltase detectable by the utilization of the hexose components by symbiotic W56, and conversely with lactase and Y87.

Use small inocula from slant-suspensions. T(μ) with .05% equiv. C-source.

W-108: ~~over~~. P23.

N24. P25 P28

glucose	-	±	+++ → M-L-	Sticks out on glu + trehalose.
fructose(st sep)	-	-	+++ → M+L+	
trehalose "	-	-	+++ → M-L-	
sucrose	-	-	-	
maltose	-	++	+++ → M+L+	
lactose	-	-	-	
Na lactate	++	+++	✓	
K gluconate	+++	+++	✓	

Y-10 glucose ++ +++.

W108

Y10

On 1% EMB plates:

N24. P25

K glucon	++	+++	+++
glucose	-	- many inclusions	+++
L-arabinose	+++	/	+++
xylose	+++	/	+++
mannitol	-	occ. wr.	++
lactose	-	"	++
maltose	-	"	+++

Look for specific phenotype inversions on glucose, maltose & lactose selections

Jan 26, 1948

Mix 1/4 ml W108 + Y10 into 1 ml (m) + .05% β galactose + .05% K-glucon. Incubate 36 hours + test for free phenol with diazo-sulfanilic reagent. (β gal gives a strong color which, however, disappears in acid solutions!). Compare with blanks, etc.:

Test 1.

1. Blank	-	-
2. Blank medium (β gal)	-	-
3. β gal .02%	+++	++++
4. 108 a	\pm	+
5. 108 b	++	+
6. Y10 c	\pm	+
7. Y10 d	++	+
8. Y10 glucose only.	-	-

Not even nearly complete splitting by either Y10 or W108 under these conditions - streak out 108 on lactose plate to assure non-reverser.

Some splitting is evident - ca. 10%.

Cross adaptation tests.

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Jan 28-9, 1948

a b c d e f.
Glucone Galactose Gluconic d-arab l-arab d-xyl.

		a	b	c	d	e	f.
W10	A Glucose	++ ✓ ++	± ± +	± ± +	- - -	- - +	- -
W10	B Galactose	++ ++ ++	++ ++ ++	- + -	- - -	++ ++ ++	- -
W108 ¹	C Gluconic ac.	+++ +++ ++	- + -	+++ +++ ++	- + -	- + +	- -
W108 ¹	D d-arabinose	± ± +++	- + -	± ± -	- - -	- - +	- -
W10	E l-arabinose	++ ++ ++	≠ ++ ++	- + -	- - -	++ ++ ++	- -
W108 ¹	F d-xylene	± ± ++	- + -	- ± -	- - -	- ± -	± ++ ++

No fum.

1 hour
2 hours
4 hours.

- ① Glucone and galactose are adaptive. Also d-xylene and l-arabinose.
- ② D-arabinose is not fermented
- ③ Galactose and arabinose cross-adapt bilaterally.
- ④ The resting cell suspensions of W108! utilize glucose!!! (Repeat).

(Cells grown overnight and harvested from YP broth 50 ml + 1% sugar. Concentrate to 7 ml. Use 1 ml cells, 1 ml yeast buffer + 1% sugar.)

→ found to be mostly blue + reversion.

Cross-adaptation tests.

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January 30, 1978.

	A'	B//	C,	D //	E
Grown in:	Glucose	Galactose	Glucosidic Arabinose	HDP.	
1. Y10	Glucose	+++ ±	+	- ±	-
2.	Galactose	± ++	++	±	-
3.	Glucosidic Arabinose	+++ ±	+	+++ ±	-
4.	l-Arabinose	± ++	++	-	++
5.	W108	-	-	-	-
6. *	Glucose	- - - - -	- - -	- - -	cells OK
7. *	Galactose	± +	++ ±	- +	cells OK
8.	Glucosidic Arabinose	-	8 -	+ +++	cells OK
9. *	l-Arabinose	± ±	++	-	cells OK, galactose by W108
10.	-	-	-	-	-
11.	-	-	-	-	-
12.	-	-	-	-	-

may be too
readily buffered.

Design as above. Cells added 11:30 AM. Variable cell yields!

2 h. 3 h.

* streak out on maltose or glucose

- ① Confirm cross-adaptation of galactose & arabinose
- ② Glucose is adaptive. Glucosidic arabinose is lacking in glucose adapted cells.

W108 - C source characterization

T(m) + .05% C source.

W108	Glycose -	MDP +±	Glyc + MDP +±
Y10.	+++	++	+++

24 hours.